

FORM PTO-1390 (REV. 9-2001)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 3276.1003-000
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/019869
INTERNATIONAL APPLICATION NO. PCT/DK00/00350	INTERNATIONAL FILING DATE 29 June 2000 (29.06.2000)	PRIORITY DATE CLAIMED 29 June 1999 (29.06.1999)	
TITLE OF INVENTION BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION			
APPLICANT(S) FOR DO/EO/US Søren Blirup-Jensen and Marianne Larsen			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</p> <p>4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input type="checkbox"/> has been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>a. <input type="checkbox"/> is attached hereto.</p> <p>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input type="checkbox"/> have been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input checked="" type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11 to 20 below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>15. <input type="checkbox"/> A substitute specification.</p> <p>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</p> <p>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</p> <p>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</p> <p>20. <input checked="" type="checkbox"/> Other items or information: International Preliminary Examination Report (7 Sheets) with annex of Amended Sheets numbered as pages 21-23 (claims as amended under Article 34)</p>			

U.S. APPLICATION NO. 10/019869		INTERNATIONAL APPLICATION NO. PCT/DK00/00350		ATTORNEY'S DOCKET NUMBER 3276.1003-000	
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21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY 	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	22 - 20 =	2	x \$18.00	\$	36.00
Independent claims	2 - 3 =		x \$84.00	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+	\$280.00
TOTAL OF ABOVE CALCULATIONS =				\$	926.00
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	
SUBTOTAL =				\$	926.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	926.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$	926.00
				Amount to be refunded:	\$
				charged:	\$

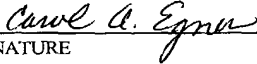
a. ☒ A check in the amount of \$ 926.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-0380. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: DAVID E. BROOK, ESQ. HAMILTON, BROOK, SMITH & REYNOLDS, P.C. 530 Virginia Road P. O. Box 9133 Concord, Massachusetts 01742	<div style="text-align: center;">  SIGNATURE Carol A. Egner NAME 38,866 REGISTRATION NUMBER </div>
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DEB/CAE/kmb
21 December 2001

10/019869

PATENT APPLICATION

Attorney's Docket No.: 3276.1003-000

10/019869
531 Rec'd PCT/P 24 DEC 2001

IN THE UNITED STATES RECEIVING OFFICE (RO/US)

Designated/Elected Office (DO/EO/US)

U.S. National Phase of

International Application No.: PCT/DK00/00350
International Filing Date: 29 June 2000
Earliest Priority Date: 29 June 1999
Applicants: Søren Blirup-Jensen and Marianne Larsen
Title: BENZOATE BUFFERS FOR ZONE
ELECTROPHORESIS AND IMMUNOFIXATION
Attorney's Docket No.: 3276.1003-000

Date: 24 December 2001
EXPRESS MAIL LABEL NO. EV005369282US

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows:

In the Specification

At page 1, following the title, please insert the following paragraph:

--RELATED APPLICATIONS

This application is the U.S. National Phase of International Application No. PCT/DK00/00350, filed 29 June 2000, designating the United States and claiming the benefit under 35 U.S.C. § 119 to Denmark Patent Application No. PA 1999 00935, filed 29 June 1999.--

In the Claims

Please amend Claims 3-10, 13-15 and 20-22 to read as follows. Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages i-ii).

3. (Amended) Buffer according to claim 2, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.
4. (Amended) Buffer according to claim 2, wherein the salt of benzoic acid is the sodium salt.
5. (Amended) Buffer according to claim 1, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
6. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L.
7. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L.
8. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L.
9. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L.

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10. (Amended) Buffer according to claim 1, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
13. (Amended) Use according to claim 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.
14. (Amended) Use according to claim 12, wherein the salt of benzoic acid is the sodium salt.
15. (Amended) Use according to claim 11, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
20. (Amended) Use according to claim 11, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
21. (Amended) Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in claim 1.
22. (Amended) Kit for zone electrophoresis and/or immunofixation comprising gels containing the buffer as defined in claim 1, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

REMARKS

This application is the United States national application arising from International Application No. PCT/DK00/00350.

Claims 3-10, 13-15 and 20-22 of the International Application have been amended to eliminate multiple dependencies.

For the convenience of the Examiner, a complete set of the new claims is attached to this Amendment under the heading "Claims Pending, as Amended Herein."

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Applicants' Attorney hereby authorizes the Patent Office to charge any additional fees to Deposit Account No. 08-0380. A copy of this letter is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Carol A. Egner
Carol A. Egner
Registration No. 38,866
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Dated: *December 24, 2001*

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531 Rec'd PCT/F 24 DEC 2001

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

3. (Amended) Buffer according to claim [1 or] 2, [comprising] wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt [of benzoic acid].
4. (Amended) Buffer according to [any one of claims 1-3, comprising] claim 2, wherein the salt of benzoic acid is the sodium salt [of benzoic acid].
5. (Amended) Buffer according to [any one of claims 1-4] claim 1, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
6. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L [in the gel buffer].
7. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L [in the gel buffer].
8. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L [in the compartment buffer].
9. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L [in the compartment buffer].

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10. (Amended) Buffer according to [any one of claims 1-9] claim 1, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
13. (Amended) Use according to claim [11 or] 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.
14. (Amended) Use according to [any one of claims 11-13] claim 12, wherein the salt of benzoic acid is the sodium salt.
15. (Amended) Use according to [any one of claims 11-14] claim 11, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
20. (Amended) Use according to [any one of claims 11-19] claim 11, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
21. (Amended) Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in [any one of claims 1-10] claim 1.
22. (Amended) Kit [according to claim 21 further] for zone electrophoresis and/or immunofixation comprising gels containing the buffer as defined in [any one of claims 1-10] claim 1, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

U.S. National Phase of PCT/DK00/00350

CLAIMS PENDING, AS AMENDED HEREIN

1. Zone electrophoresis and/or immunofixation buffer comprising benzoic acid and/or a salt thereof.
2. Buffer according to claim 1, comprising a salt of benzoic acid.
3. (Amended) Buffer according to claim 2, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.
4. (Amended) Buffer according to claim 2, wherein the salt of benzoic acid is the sodium salt.
5. (Amended) Buffer according to claim 1, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
6. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L.
7. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L.
8. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L.

9. (Amended) Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L.
10. (Amended) Buffer according to claim 1, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.
12. Use according to claim 11, wherein a salt of benzoic acid is used.
13. (Amended) Use according to claim 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.
14. (Amended) Use according to claim 12, wherein the salt of benzoic acid is the sodium salt.
15. (Amended) Use according to claim 11, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.
17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, 4.5 to 5 g/L in the compartment buffer.
19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.
20. (Amended) Use according to claim 11, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
21. (Amended) Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in claim 1.
22. (Amended) Kit for zone electrophoresis and/or immunofixation comprising gels containing the buffer as defined in claim 1, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

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BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION

The present invention relates to buffers comprising benzoic acid and/or a salt thereof for use in zone electrophoresis and/or immunofixation. The invention further relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation. The invention also concerns kits for zone electrophoresis and/or immunofixation. An optimised combination of the gel buffer and the compartment buffer is disclosed herein giving sharper and easier identifiable protein bands or protein pattern. A special advantage being that the buffers disclosed herein are non-hazardous.

15

BACKGROUND OF THE INVENTION

The principle of immunofixation was described by Alfonzo and Wilson in 1964 (ref. 1). The method was later modified by Alper and Johnson in 1969 and used for identification of genetic protein variants (ref. 2). Immunofixation is a widely used diagnostic method. It is a rapid, important and useful tool for the examination and identification of various protein abnormalities in serum, urine, cerebrospinal and synovial fluids.

The immunofixation procedure can be used for the identification of any single protein band of an electrophoresis. The technique is a combination of zone electrophoresis followed by immunofixation using monospecific antibodies. In this way it is possible to separate and identify different proteins in a biological mixture according to their physicochemical properties and antigenic properties.

35

The immunofixation procedure is most frequently used for the detection of monoclonal immunoglobulins in serum and Bence Jones proteins in urine.

5 Usually, barbital buffers comprising barbituric acid and/or sodium barbiturate are used (ref. 3). In fact, this use is recommended as barbituric acid/sodium barbiturate provide a good separation of all protein bands. However, barbituric acid and sodium barbiturate
10 are hazardous compounds which potentially cause irritation by contact with the skin, the eyes or the respiratory system, and which in extreme cases even may cause death. In more and more countries, the use of barbituric acid and sodium barbiturate in buffers is
15 therefore prohibited.

Thus, buffer components which can replace barbituric acid and sodium barbiturate and which further possess the advantages of barbituric acid and/or sodium barbiturate
20 are still needed. The present invention provides such compounds which can replace barbituric acid and sodium barbiturate.

SUMMARY OF THE INVENTION

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In a first aspect, the present invention relates to buffers comprising benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation.

30 In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation.

In a third aspect, the present invention concerns kits for zone electrophoresis and/or immunofixation comprising a buffer as described herein.

- 5 The present invention is described in detail in the following.

BRIEF DESCRIPTION OF THE FIGURE

- 10 Figure 1 shows an immunofixation gel obtained following the procedures described in Example 1.

Figures 2A, 2B, 2C and 2D show four patient serum samples subjected to zone electrophoresis and immunofixation with
15 the buffer of the invention comprising the sodium salt of benzoic acid.

Figures 3A, 3B, 3C and 3D show the four patient serum samples as shown in Figures 2A, 2B, 2C and 2D subjected
20 to zone electrophoresis and immunofixation with the buffer of the invention comprising the ammonium salt of benzoic acid.

DETAILED DESCRIPTION OF THE INVENTION

25

The present invention relates buffers for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.

- 30 From Chromatographia Vol. 48, No. 5/6, 383-387 (1998) (ref. 4), Deutsche Lebensmittel-Rundschau 94. Jahrg., Heft 1, 28-30 (1998) (ref. 5), Commun. Soil Sci. Plant Anal. 30 (1 & 2), 213-220 (1999) (ref. 6), Journal of Chromatography A. 781, 497-501 (1997) (ref. 7), Journal
35 of Pharmaceutical and Biomedical Analysis 15, 63-71 (1996) (ref. 8), and J. Cap. Elec. Vol. 2, No. 5, 235-240

(1995) (ref. 9) buffers containing benzoic acid or sodium benzoate are known. The buffers are used in capillary electrophoresis for the determination of i.a. organic acids, phosphate, phytin acid, short-chain fatty acids, and cyclodextrins. The principle of capillary electrophoresis is very different from the principles of zone electrophoresis in gels and immunofixation.

From US 4,321,119 (ref. 10), a non-barbiturate buffer composition for use in the electrophoretic separation of proteins into fractions is known, said buffer comprising a water soluble salicylate such as sodium salicylate and an inorganic salt. The salicylate is used in a concentration of from 0.5 to 1 g salicylate to 1.5 to 3 g inorganic salt.

In particular, the buffer of the invention may comprise a salt of benzoic acid. Examples of suitable salts is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid. The buffer of the invention may comprise one or more of these salts of benzoic acid optionally in combination with benzoic acid itself. Accordingly, benzoic acid or one or more salts of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. In a preferred embodiment, the buffer of the invention comprises a salt of benzoic acid, in particular the sodium salt of benzoic acid.

The buffer of the invention is particularly suitable for separation of proteins, serum proteins, and immunoglobulins in zone electrophoresis and/or immunofixation. Proteins are large molecules which are susceptible to denaturation, thus, making them sensitive to the conditions employed in the separation procedure. E.g. heat development during the electrophoresis

procedure may result in destruction of the protein structure. Increased ionic strength of the running buffer increases the electric conductivity, thus leading to an increased heat development. This can be avoided e.g. by
5 using heavy cooling procedures during the electrophoresis procedure. Alternatively, low concentration of ion-providing components of the running buffer can be used. For instance, in US 4,321,119, a relatively low concentration of salicylate is used.

10

However, it has surprisingly been found that a buffer comprising benzoic acid and/or a salt thereof does not suffer from the above drawbacks. The benzoic acid and the salts thereof can be used in relatively high
15 concentrations without the need for additional cooling in order to avoid breakdown of the protein structure or denaturation. An additional advantageous feature of the buffer is that the benzoic acid and the salts thereof in general act as preserving agents. This is especially
20 observed in the case of sodium benzoate (the sodium salt of benzoic acid).

The buffer of the invention is used as a gel buffer and/or as a compartment buffer. The term "buffer"
25 includes both. The gel buffer and the compartment buffer may have the same acid/salt concentration, or the concentrations of the gel buffer and the compartment buffer may be different.

30 The gel buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L,
35 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular, the concentration of benzoic

acid and/or the salt thereof may be 7 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range from 6.5 to 7.5 g/L, more specifically around a concentration of about 7 g/L.

The compartment buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular, the concentration of benzoic acid and/or the salt thereof may be 3.5 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range 3 to 4 g/L, more specifically around a concentration of about 3.5 g/L.

It lies within the scope of the present invention to use each of the components (benzoic acid itself or any of its salts) in the concentrations specified above.

The zone electrophoresis/immunofixation procedure is a very powerful tool in the early diagnosis of various diseases. Frequently, the disease can be diagnosed even before the patient experiences symptoms of the disease. Therefore, a distinct separation of the protein bands is of crucial importance in order to make a reliable diagnosis. Especially the separation of the immunoglobulins is of major importance. These proteins are produced in the bone marrow and their appearance in immunofixation reflects the status of the bone marrow. A cancer disease in the bone marrow affecting the plasma cells may lead to changed synthesis of the immunoglobulins and thereby the appearance of these

immunoglobulins in immunofixation. The normal heterogeneous pattern is most frequently changed into a pattern of distinct bands with different mobility. In particular in such cancers an early treatment regime is important for the survival of the patient and also the treatment, e.g. chemotherapy, radiation and/or plasma-pheresis, as such influences the patient's well-being to a major extent. As evident from the Figures, the buffers of the invention provide such good and distinct separation enabling a reliable diagnosis.

The buffer of the invention may further comprise additional components such as Tris and/or Tricine and/or calcium lactate and/or sodium azide.

The benzoic acid compounds as defined above are suitable as buffer components, in particular as gel buffers and compartment buffers. They are much less hazardous than the conventionally used barbiturates. Furthermore, no hazard labelling of the buffers is required. It has further been shown (cf. Example 1) that the buffers of the invention seem to provide sharper and more well defined bands than the conventionally used barbiturate-containing buffers. Also, the use of a compartment buffer having a lower salt/acid concentration than the gel buffer may be advantageous. This may inhibit the heat development during electrophoresis and yield sharper and more well defined bands.

Furthermore, the buffer may contain one or more additional components such as buffering agents, preserving agents, colouring agents, salts, detergent and surfactants.

In a special embodiment of the buffer of the present invention, the gel buffer comprises the sodium salt of

benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 6.5 to 7.5 g/L, in particular 7 g/L.

In a special embodiment of the buffer of the present invention, the compartment buffer comprises the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 3 to 4 g/L, in particular 3.5 g/L.

In particular, the gel buffer may have a salt concentration of 7 g/L, and the compartment buffer a concentration of 3.5 g/L.

As mentioned, the buffer is for use in zone electrophoresis, and/or immunofixation. The test samples are suitably serum, urine, cerebrospinal or synovial fluids.

In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

Benzoic acid or a salt of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. Examples of suitable salts of benzoic acid are the sodium salt, the potassium salt, the calcium salt, the magnesium salt and the ammonium salt. In a preferred embodiment, the salt of benzoic acid is the sodium salt.

As mentioned above, it has surprisingly been found that benzoic acid or the salts thereof can be used in relatively high concentrations. Thus, in one embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L. In particular, in the gel buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular the concentration may be from 6.5 to 7.5 g/L, like 7 g/L. In particular, in the compartment buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular the concentration may be from 3 to 4 g/L, like 3.5 g/L.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L is used as a component of the gel buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 6.5 to 7.5 g/L, in particular a concentration of 7 g/L, is used as a component of the gel buffer.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L is used as a component of the compartment buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 4 g/L, in particular a concentration of 3.5 g/L, is used as a component of the compartment buffer.

In a further embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof in a buffer for electrophoresis and/or immunofixation, wherein the
5 buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

In third aspect, the present invention relates to kits for zone electrophoresis and/or immunofixation, which
10 kits comprise a buffer as defined above.

In one embodiment, the kit further comprises gels containing the buffer of the invention, staining solutions, antibodies (e.g. rabbit immunoglobulins),
15 blotters, templates, fixation and/or reagents.

The gel to be used is suitably an agarose gel. The agarose gel may suitably be provided in a ready-to-use packing containing the buffer of the invention. The
20 buffer of the invention may thus be used as compartment buffer as well as gel buffer for supporting medias such as agarose, starch, polyacrylamide etc.

The invention is further illustrated by the following, non-limiting example.
25

EXAMPLES

EXAMPLE 1

30

Materials. Agarose Gel, 10 plates (gel buffer). Ready-to-use. Each plate is 8.3×10.2 cm and contains on a transparent, flexible plastic backing, agarose gel containing the buffer of the invention comprising sodium
35 benzoate (i.e. sodium salt of benzoic acid) or ammonium

benzoate (i.e. the ammonium salt of benzoic acid) (1% gel, 99% buffer) preserved with sodium azide.

5 Concentrated Buffer. 3x75 mL (13.33 x concentrated)
buffer of the invention preserved with sodium azide. The
content of each of the bottles of buffer is diluted prior
to use to a total volume of 1000 mL with distilled water.
The diluted buffer contains sodium benzoate (3.5 g/L) or
10 ammonium benzoate (3.5 g/L), Tris (3.6 g/L), Tricine (0.6
g/L), calcium lactate (0.75 g/L), and sodium azide (0.04
g/L).

15 Concentrated Staining Solution. 75 mL (4 x concentrated).
Amido Black in 5% acetic acid. The Staining Solution is
diluted prior to use to a total volume of 300 mL with
distilled water. The concentration of Amido Black in the
diluted Solution is 5 g/L.

20 Test Sample. Serum samples, optionally freshly drawn. 33
samples were tested.

Sample Template. 10 pieces.

25 Antibody Template. 10 pieces.

Gel Blotter. Pre-cut disposable, filter paper, 1 package,
40 sheets.

30 Sample Blotter. Pre-cut disposable, filter paper, 1
package, 10 sheets.

Drying Blotter. Pre-cut disposable filter paper, 2
package, 20 sheets each.

Fixation Reagents. Protein Fixative Solution 1.0 mL containing 7% sulphosalicylic acid and 5% acetic acid. Green dyed.

- 5 Rabbit Anti-Human IgG. Specific for γ -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

- 10 Rabbit Anti-Human IgA. Specific for α -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

- 15 Rabbit Anti-Human IgM. Specific for μ -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

- 20 Rabbit Anti-Human Kappa Light Chains. Specific for kappa light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

- Rabbit Anti-Human Lambda Light Chains. Specific for lambda light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

- 25 Other reagents. Saline Solution (0.9% NaCl). For dilution of the samples and washing of the gel. Destaining Solution (acetic acid, 5%). Distilled or deionised water.

- 30 Equipment. Power supply 120 V constant. Electrophoresis apparatus for Agarose Gels (DAKO Electrophoresis Apparatus Code No. S 2200). Pipettes (5 μ L, 80 μ L). Containers for washing, staining and destaining of Agarose Gels (DAKO Washing and Staining Accessory Kit Code No. S 2201). Glass plate (minimum 11x11 cm) plus a weight of approximately 1 kg for pressing the gel.
35 dryer or a drying oven (maximum 90°C).

- Additional Reagents. Rabbit Anti-Human IgD (DAKO Code No. A 0093), specific for δ -chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human IgE
- 5 (DAKO Code No. A 0094), specific for ϵ -chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human Kappa Free Light Chains (DAKO Code No. A 0100), specific for kappa free light chains, immunoglobulin fraction, preserved with 15 mM sodium
- 10 azide. Rabbit Anti-Human Lambda Free Light Chains (DAKO Code No. A 0101), specific for lambda free light chains, immunoglobulin fraction, preserved with 15 mM sodium azide.
- 15 Preparation of specimens. All serum specimens should preferably be diluted with saline solution just prior to use. For the reference pattern, serum should be diluted 1:4 (1 part serum + 3 parts Saline Solution). For the immunofixation patterns serum should be diluted 1:15 (1
- 20 part serum + 14 parts saline solution). For serum suspected of containing low levels of monoclonal immunoglobulins, a dilution of 1:4 is recommended. For serum specimens suspected of containing high levels of monoclonal immunoglobulin (>30 g/L), a dilution of 1:31
- 25 may be suitable.

For the detection of Bence Jones proteins in urine, the urine sample should be concentrated (e.g. by ultrafiltration) to a total protein concentration of at

30 least 1 g/L. This concentrated urine is applied in all slots. The light chain antibodies as described above will precipitate kappa or lambda chains whether they are free or still part of the immunoglobulin molecule. In order to determine if detected light chains are present as free

35 light chains in the urine, special antibodies as

described above against free kappa and free lambda light chains could be employed.

Assay procedure

- 5 Zone Electrophoresis (separation of the proteins). All samples are prepared as described above. The Agarose Gel is removed from the foil package and placed on a level surface. Excess moisture is removed from the gel surface by gentle blotting with a Gel Blotter. The Sample
- 10 Template is placed on the surface of the gel so that the slots are in alignment with the arrows located on the edges of the gel. 5 μ L of the pre-diluted serum sample is applied across each slot. The 1:4 serum dilution is applied in the slot marked Ref., and the 1:15 serum
- 15 dilution in the other 5 slots. The sample is allowed to diffuse into the gel for 5 minutes, and then the sample template is blotted gently with a Sample Blotter in order to remove excess sample liquid. The Blotter is discarded, and the Sample Template is carefully removed and
- 20 discarded.

- Electrophoresis. The DAKO Electrophoresis Apparatus is filled with 300 mL diluted buffer (150 mL in each compartment). The gel is placed in the apparatus so as to
- 25 form an arch (gel side down) in such a way that the (-) side of the gel dips into the cathode compartment (-), and that the (+) side of the gel dips into the anode compartment (+). The lid is placed on the apparatus and power supply is connected. The voltage is set to 120 V
- 30 and the electrophoresis is continued for 25 minutes. Upon completion of the electrophoresis, the power supply is disconnected, and the gel is carefully removed from the apparatus and placed on a level surface, gel side up. The electrophoresis buffer is discarded.

Immunofixation (specific precipitation of the separated proteins). The surface of the gel is gently blotted with a Gel Blotter. The Gel Blotter is removed immediately and discarded. The Antibody Template is placed over the surface of the gel so that the troughs of the Template are in alignment with those printed on the plastic backing of the gel. It should be ensured that a close contact between the Template and the surface of the gel is obtained. The Template is gently rubbed in order to remove air bubbles. The following is applied: Ref.: 80 µL of Protein Fixative Solution, IgG: 80 µL of Anti-IgG, IgA: 80 µL of Anti-IgA, IgM: 80 µL of Anti-IgM, K: 80 µL of Anti-Kappa, and L: 80 µL of Anti-Lambda. It should be ensured that the volume is evenly distributed within the trough. Furthermore, the surface of the gel should not be touched. The gel is incubated with the Antibody Template in a humid box for 15 minutes at room temperature. Subsequently, the gel is placed on a levelled surface and the Antibody Template is carefully removed.

Pressing, washing, staining, destaining and drying (removal of non-precipitated proteins and staining of the precipitated protein bands). The containers of the DAKO Washing and Staining Accessory Kit are filled in the following way: Washing: 1 container with 300 mL Saline Solution. Staining: 1 container with 300 mL diluted Staining Solution. Rinsing: 1 container with 300 mL distilled water. Destaining: 1 container with 300 mL acetic acid, 5%. The gel is covered with one sheet of Gel Blotter, two sheets of Drying Blotter and a glass plate. The gel is pressed under a weight of approximately 1 kg for 10 minutes. Subsequently, the Blotters are removed and discarded, and the gel is immersed in saline solution and washed for 10 minutes without agitation. The pressing procedure is repeated as previously described. After pressing, the Blotters are discarded, and the gel is

dried in a current of hot air or, alternatively, dried in a drying oven (maximum temperature 90°C) for approximately 5 minutes.

- 5 Staining is performed for 5 minutes in the Diluted Staining Solution.

Excess Staining Solution is rinsed off in distilled water before destaining. Destaining is performed in fresh
10 Destaining Solution for approximately 2 minutes, or until the background has a faint blue colour.

Finally, the gel is dried for 5 minutes as previously described or until the gel is completely dry.

15 In Figures 1, 2 (A, B, C and D) and 3 (A, B, C and D) the results obtained are shown. Each immunofixation gel shows the results of one patient serum sample. The lane at the left is a reference lane showing all serum proteins in the serum sample in the order albumin (top), followed by
20 alpha, beta, and gamma globulins (immunoglobulins). The following lanes are a visualisation of the patient's immunoglobulins established by the use of specific antibodies. From left to right, the gel shows IgG, IgA, IgM, kappa light chain, and lambda light chain.
25

The results shown in Figure 1 are obtained using the sodium benzoate buffer.

30 The results shown in Figure 2 are obtained using the sodium benzoate buffer. Figure 2A shows a patient having clearly defined double bands in the gamma region. The bands can be identified as IgM, kappa. Figure 2B shows a patient having strong double bands in the gamma region.
35 The bands are stronger than in Figure 2A. The bands can be identified as IgM, kappa. Figure 2C shows a patient

having a strong band which can be identified as IgA, lambda. Figure 2D shows a patient having a very strong band which can be identified as IgG, lambda.

- 5 The results shown in Figure 3 are obtained using the ammonium benzoate buffer. Figures 3A, 3B, 3C and 3D shows the serum samples as in Figures 2A, 2B, 2C and 2D. The only difference being that the buffer used is an ammonium benzoate buffer. As appears from the figure, the results
10 obtained using the ammonium benzoate buffer are not as excellent as the results obtained using the sodium benzoate buffer. However, this might be due to lack of optimisation.
- 15 Results. The overall function of the buffer is comparable to conventional barbiturate-containing buffer (DAKO Code No. K 0390). Furthermore, sharper and more well defined bands were obtained. A further advantage of the novel buffer is that it is non-hazardous.

20

EXAMPLE 2

- A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-
25 up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate was varied between 1 and 10 g/L, and the
30 compartment buffer content of sodium benzoate was varied accordingly. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

35

g/L sodium benzoate in gel buffer	Performance
1	1
2	1
3	1
4	2
5	2
7	3
10	1

As it appears from the table, it seems as if the performance of sodium benzoate is peak-like around a concentration maximum of about 7 g/L. Of course this peak may be shifted depending on the nature and characteristics of the other components of the buffer.

EXAMPLE 3

10

A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate being 7 g/L, whereas the compartment buffer content of sodium benzoate was varied between 0.8 and 7 g/L. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

15

20

g/L sodium benzoate in compartment buffer	Performance
0.8	1
1.2	1
1.8	2
3.5	3
4.7	3
7	2

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28 June 2001

Patent Application No. PCT/DK00/00350 - PCT

5 REVISED CLAIMS

1. Zone electrophoresis and/or immunofixation buffer comprising benzoic acid and/or a salt thereof.
- 10 2. Buffer according to claim 1, comprising a salt of benzoic acid.
3. Buffer according to claim 1 or 2, comprising the sodium salt, the potassium salt, the calcium salt, the
15 magnesium salt and/or the ammonium salt of benzoic acid.
4. Buffer according to any one of claims 1-3, comprising the sodium salt of benzoic acid.
- 20 5. Buffer according to any one of claims 1-4, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
- 25 6. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.
- 30 7. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.
- 35 8. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L,

3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

5 9. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

10 10. Buffer according to any one of claims 1-9, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

15 11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

12. Use according to claim 11, wherein a salt of benzoic acid is used.

20 13. Use according to claim 11 or 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.

25 14. Use according to any one of claims 11-13, wherein the salt of benzoic acid is the sodium salt.

30 15. Use according to any one of claims 11-14, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

35 16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

5 18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

10

19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

15 20. Use according to any one of claims 11-19, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

20 21. Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in any one of claims 1-10.

25 22. Kit according to claim 21 further comprising gels containing the buffer as defined in any one of claims 1-10, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

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(71) Applicant (for all designated States except US): **DAKO**
A/S [DK/DK]; Produktionsvej 42, DK-2600 Glostrup
(DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BLIRUP-JENSEN**,
Søren [DK/DK]; Linde Allé 49, DK-2750 Ballerup
(DK). **LARSEN, Marianne** [DK/DK]; Jerismosevej 79,
DK-2670 Greve (DK).

(74) Agent: **HOFMAN-BANG A/S**; Hans Bekkevolds Allé 7,
DK-2900 Hellerup (DK).

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(54) Title: **BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION**

(57) Abstract: Buffers comprising benzoic acid and/or a salt thereof are disclosed, which buffers are suitably for use for zone electrophoresis and/or immunofixation. Furthermore, the use of benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation is disclosed. Kits for zone electrophoresis and/or immunofixation are also provided.

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Fig. 1

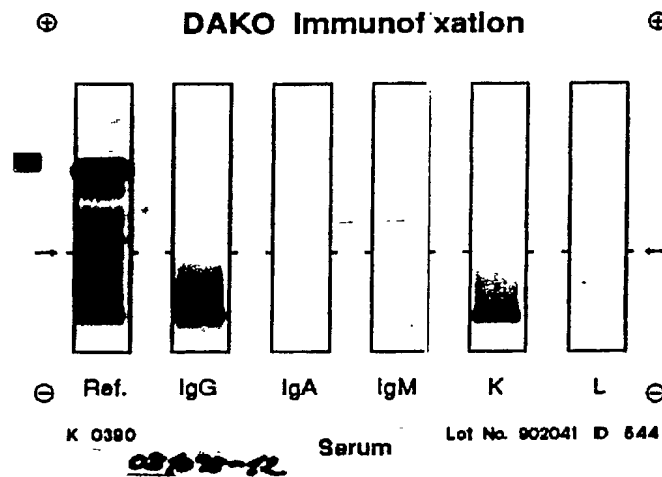


Fig. 2A

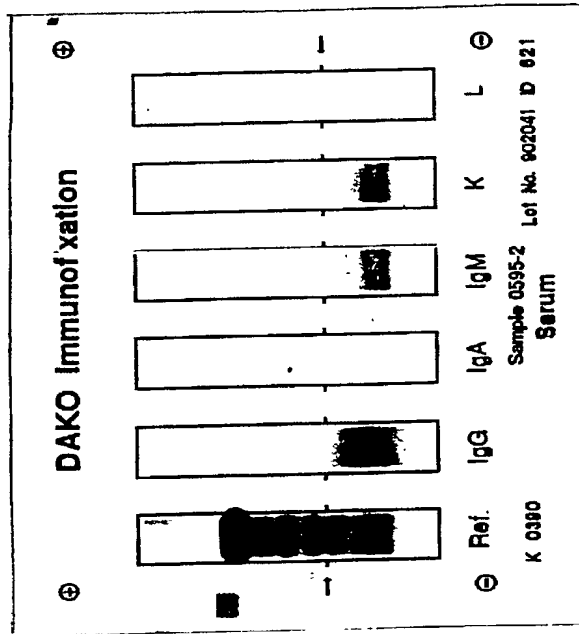


Fig. 2B

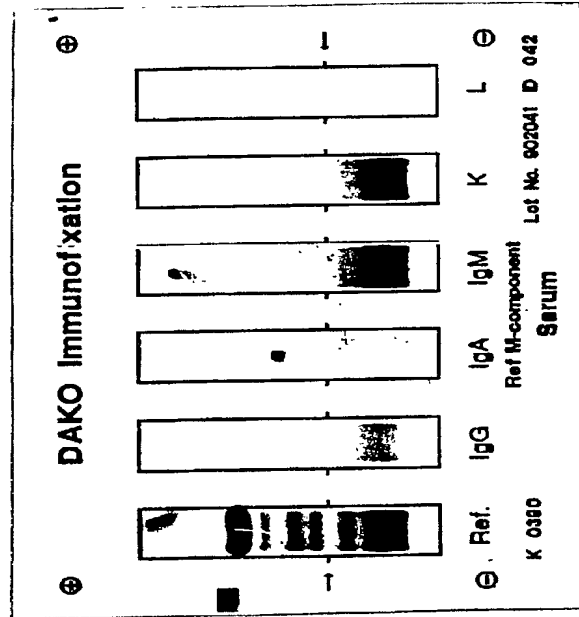


Fig. 2D

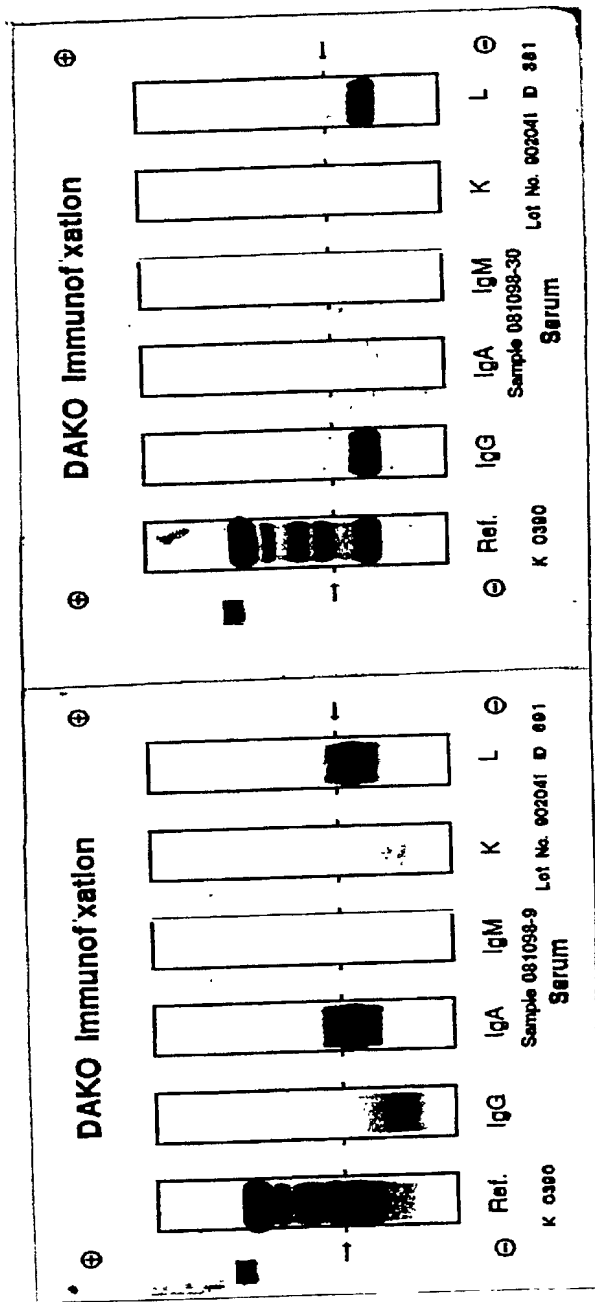


Fig. 2C

4/5

Fig. 3A

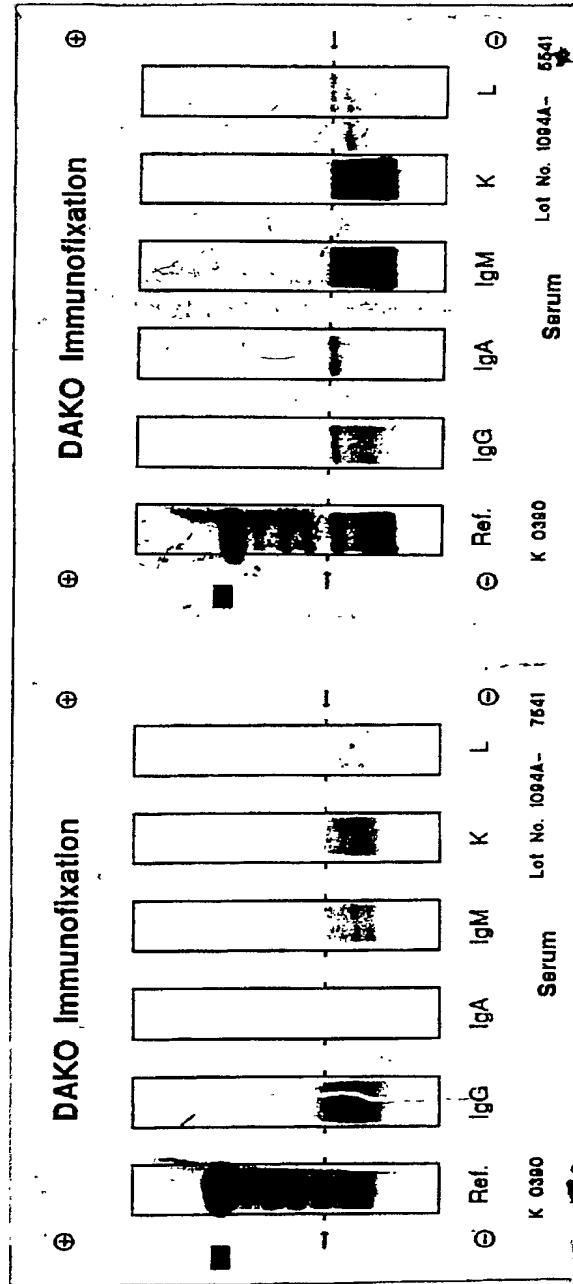
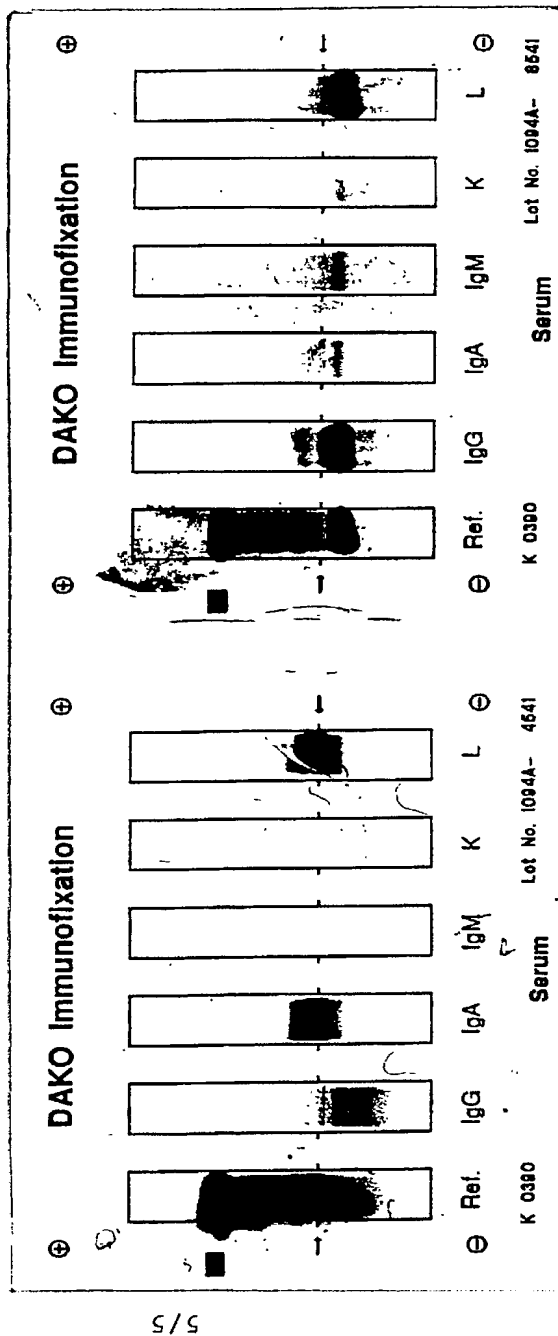


Fig. 3B

SUBSTITUTE SHEET (RULE 26)

Fig. 3C

Fig. 3D



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Declaration for Patent Application

[] Supplemental (37 C.F.R. §1.67)

As a named inventor, I hereby declare that:

My residence, mailing address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed) or an original, first and joint inventor (if plural names are listed in the signatory page(s) commencing at page 2 hereof) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Benzoate Buffers for Zone Electrophoresis and Immunofixation

the specification of which (check one)

[] is attached hereto.

[X] was filed on December 24, 2001 as United States Application Number 10/019,869, as the U.S. National Phase of PCT International Application No. ✓ PCT/DK00/00350, filed on 29 June 2000, which was amended on 28 June 2001. The U.S. National Stage Application was amended on December 24, 2001 by Preliminary Amendment filed concurrently with the U.S. National Phase Application, under Express Mail Label EV 005369282 US.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119 or 365 of any foreign application(s) for patent or inventor's certificate, or of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

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				YES	NO
<u>✓ PA 1999 00935</u> (Number)	<u>✓ Denmark</u> (Country)	<u>✓ 29 June 1999</u> (Day/Month/Year filed)	[]	[X]	[]
<u> </u> (Number)	<u> </u> (Country)	<u> </u> (Day/Month/Year filed)	[]	[]	[]
<u> </u> (Number)	<u> </u> (Country)	<u> </u> (Day/Month/Year filed)	[]	[]	[]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole

or first inventor Søren Blirup-Jensen

Inventor's Signature *Søren Blirup-Jensen* Date 2002-03-22

Residence Linde Allé 49

DK-2750 Ballerup, Denmark DKX

Citizenship Denmark

Mailing Address Same as above

Full name of second joint

inventor, if any Marianne Larsen

Inventor's Signature *Marianne Larsen* Date 2002-03-22

Residence Jerismosevej 79

DK-2670 Greve, Denmark DKX

Citizenship Denmark

Mailing Address Same as above

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HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

**POWER OF ATTORNEY OR
AUTHORIZATION OF AGENT AND
CORRESPONDENCE ADDRESS**

Application Number	10/019,869
Filing Date	29 June 2000
First Named Inventor	Blirup-Jensen
Group Art Unit	Not assigned
Examiner Name	Not assigned
Attorney Docket Number	3276.1003-000

I/We hereby appoint

☒ the attorneys/agents associated with **Customer No. 021005**

☐ Practitioner(s) named below:

as my/our attorneys/agents to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

The correspondence address for the above-identified application is:

☒ Customer Number 021005

Hamilton, Brook, Smith & Reynolds, P.C.

530 Virginia Road

P.O. Box 9133

Concord, Massachusetts 01742-9133

☐ Other

Please direct all telephone calls and facsimiles to:

Name David E. Brook, Esq. Tel. No. 978-341-0036 Fax No. 978-341-0136

I am the:

☐ Applicant/Inventor.

☒ Authorized representative of the Assignee of the entire interest. See 37 C.F.R. 3.71. A Statement under 37 C.F.R. §3.73(b) is enclosed.

☐ Authorized representative of an assignee together with ☐ of the entire interest. A separate Statement under 37 C.F.R. § 3.73(b) is enclosed.

SIGNATURE of Applicant or Assignee of Record

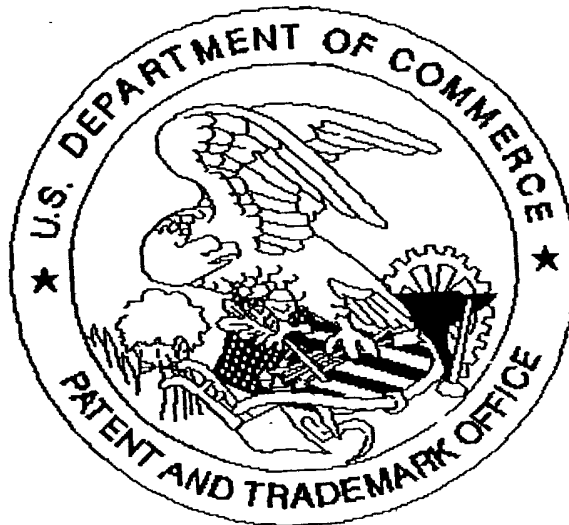
Name Marianne Hundewadt Christensen, IPR Counsellor

Signature Marianne Hundewadt Christensen

Date 2 April 2002

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